Institute Report No. 267

Acute Oral Toxicity of Guanidine Nitrate in Rats

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

May 1988

Toxicology Series: 81

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute oral toxicity of guanidine nitrate in rats (Toxicology Series 81)--Mullen et al.

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Edwin S. Beatrice 4 May 88

Edwin S. Beatrice (date)
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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No. 267	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Branch Division of Toxicology 6b OFFICE SYMBOL (If applicable) SGRD-UL-TO-M	7a NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5010
8a NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62720A; PROJECT NO: 835; TASK NO: AB; WORK UNIT ACCESSION NO: DA 303913

11 TITLE (Include Security Classification)
Acute Oral Toxicity of Guanidine Nitrate in Rats

12 PERSONAL AUTHOR(S)
Mullen L, Morgan EW, Lewis CM, Wheeler CR, and Korte DW Jr

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Institute	FROM _____ TO _____	May 1988	64

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Acute Oral Toxicity, Guanidine Nitrate, Nitroguanidine, Munition, Rat

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of guanidine nitrate was determined in male and female albino Sprague-Dawley rats administered a single dose by oral gavage. The median lethal doses (MLD) were 989.6±68.7 mg/kg in male and 729.8±34.3 mg/kg in female rats. The primary category of clinical signs was behavioral (e.g., inactive, irritable, disoriented, hyperactive, ataxic) which was observed in 63 of 99 animals dosed with guanidine nitrate. Other categories of frequently observed clinical signs were gastrointestinal (e.g., material in mouth, perianal staining, increased salivation, diarrhea) which was observed in 37 of 99 animals, and respiratory (e.g., reddish nasal discharge, increased rate and/or decreased depth of respiration) which was observed in 26 of 99 animals. These findings suggest that guanidine nitrate is a slightly toxic compound with a primary effect on the central nervous/neuromuscular system.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	unclassified
22a NAME OF RESPONSIBLE INDIVIDUAL Edwin S. Beatrice, COL, MC	22b TELEPHONE (Include Area Code) 415 - 561-3600 22c OFFICE SYMBOL

DD Form 1473, JUN 86 *Previous editions are obsolete*

ABSTRACT

The acute oral toxicity of guanidine nitrate was determined in male and female albino Sprague-Dawley rats administered a single dose by oral gavage. The median lethal doses (MLD) were 989.6 ± 68.7 mg/kg in male and 729.8 ± 34.3 mg/kg in female rats. The primary category of clinical signs was behavioral (e.g., inactive, irritable, disoriented, hyperactive, ataxic) which was observed in 63 of 99 animals dosed with guanidine nitrate. Other categories of frequently observed clinical signs were gastrointestinal (e.g., material in mouth, perianal staining, increased salivation, diarrhea) which was observed in 37 of 99 animals, and respiratory (e.g., reddish nasal discharge, increased rate and/or decreased depth of respiration) which was observed in 26 of 99 animals. These findings suggest that guanidine nitrate is a slightly toxic compound with a primary effect on the central nervous/neuromuscular system.

Key Words: Acute Oral Toxicity, Guanidine Nitrate, Nitroguanidine, Munition, Rat

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT/APC: WU 180, Environmental Health Effects of Army Materials/APC: TLB0

GLP STUDY NUMBER: 84001

STUDY DIRECTOR: MAJ Don W. Korte, Jr, PhD, MSC
Chief, Division of Toxicology

PRINCIPAL INVESTIGATOR: Lawrence Mullen, BS, SP5

CO-PRINCIPAL INVESTIGATOR: Carolyn M. Lewis, MS

PATHOLOGIST: LTC Lance O. Lollini, DVM, VC, Diplomate, American College of Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Guanidine Nitrate

INCLUSIVE STUDY DATES: 29 February - 11 April 1984

OBJECTIVE: To determine the acute oral toxicity of guanidine nitrate in Sprague-Dawley rats.

ACKNOWLEDGMENTS

SP5 Thomas P. Kellner, BA, and SP4 Steven K. Sano, BS, provided research assistance; Richard D. Spieler, Susan Hernandez, and Michael Sands provided animal care and facility management; and Callie B. Crosby, MA, and Brenda Goce provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, declare that GLP Study 84001 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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Study Director

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CPT, VC
Co-Author

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 22 April 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for GLP Study 84001

1. I hereby certify that in relation to LAIR GLP Study 84001, the following inspections were made:

14 March 1984 - Dosing and Observations

2. The report and raw data for this study were audited on 21 April 1987.

Walter G. Bell
WALTER G. BELL
SFC, USA
Quality Assurance Auditor

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Acute Oral Toxicity of Guanidine Nitrate in Rats--
Mullen *et al*

INTRODUCTION

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, reviewed the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of the Study

The objective of this study was to determine the acute oral toxicity of guanidine nitrate in Sprague-Dawley rats.

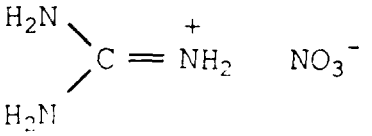
MATERIALS

Test Substance

Chemical name: Guanidine Nitrate

Chemical Abstracts Service Registry Number: 506-93-4

Structural formula:



Molecular formula: $CH_6N_3 \cdot NO_3$

Additional chemical information appears in Appendix A.

Vehicle

The vehicle consisted of 0.2% methylcellulose (Sigma, St. Louis, MO, Lot Number 72F-0478), 0.4% Tween® 80 (Fisher Scientific, Fairlawn, NJ, Lot Number 713137), and sterile water for injection (Cutter Medical, Berkeley, CA, Lot Number 426-27, 13 Mar 84, Lot Number CH7936, 20 Mar 84).

Animal Data

Fifty-five male and 63 female Sprague-Dawley rats (Harlan-Kingman Inc, Fremont, CA) were used for this acute oral toxicity study. Both males and females were within acceptable body weight ranges upon receipt (123 to 178 g). The right ear of each rat was tagged for identification, and their cages were labeled with the corresponding number. Additional animal data appear in Appendix B.

Husbandry

The rats were housed individually in stainless steel, wire mesh cages with automatically flushing dumptanks. The diet consisted of Purina Certified Rodent Chow No. 5002 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by automatic lick dispensers. The temperature and relative humidity of the animal rooms were constantly monitored and maintained at 22.8° to 25.0°C and 40% to 52% (with a temporary spike up to 64%) for males housed in room B[illegible]418. The temperature and relative humidity for females housed in B[illegible]418 were 20.6° to 22.8°C and 44% to 64% (with a temporary drop up to 74%). An additional group of females was housed in B31409. The temperature and relative humidity were maintained at 22.2° to 26.7°C (with a temporary drop to 18.3°C) and 32% to 44%. The photoperiod was 12 hours of light per day.

METHODS

Acclimation and Study Assignment

After seven days of quarantine for males and six days for females, the rats were assigned to the various dose groups using the ASYS® Animal Allocation System in accordance with [illegible]-[illegible]-84, "Standard Procedures for Assigning Animals to Treatment Groups." Ten animals were assigned to each dose group, with the exception of group 6A, which was assigned eight animals, and the vehicle control group, which was assigned five animals.

Compound Preparation

Depending upon the dose level, various amounts of guanidine nitrate were weighed in a Mettler AK 160 Electronic Balance and were added to 50 ml of the vehicle for males and 25 ml of the vehicle for females to yield the desired dosing concentration. Complete analyses of the dosing solutions/suspensions for homogeneity and verification of concentrations appear in Appendix A.

Dose Levels

The Approximate Lethal Dose (ALD) was 750 to 1000 mg/kg for both male and female rats. Based on these data, test dosages were selected (Table 1). The 610-mg/kg dose level was added to the female dose groups to define the lower limits of the dose-response curve. A data point below 50% mortality is necessary to obtain a proper statistical derivation of the MLD value.

TABLE 1

Guanidine Nitrate Dosages (mg/kg)

Males	Females
683*	610
826	718
1000	847
1210	1000
1470	1180
	1390

* By chemical analysis of this solution it was determined that these animals actually received 311 mg/kg.

Test Procedure

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3). All doses were calculated by using a program developed for the Hewlett-Packard 9815A programmable calculator. The volumes administered ranged from 1.9 to 2.6 ml for males and 1.2 to 1.8 ml for females depending on the animal's weight and dose

group. Each rat in the vehicle control group received 2.0 ml. All dosing material was administered with Perfektum® (Popper & Sons, Inc., New Hyde Park, NY) stainless steel animal feeding tubes. Before each dosing, the suspension was vortexed to ensure adequate distribution. The volume of each dose was drawn from the middle of each test chemical vial with 3.0-ml plastic, disposable, sterile syringes. The dosing procedures were conducted without animal sedation or anesthesia.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. At least one observation was recorded during the first 4 hours after dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after termination by barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. Selected lethal dose values were derived by probit analysis according to the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to determine the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Deviations from Protocol

Due to the length of time required to complete dosing of the animals, the multiple observations scheduled for the first 4 hours after dosing could not be accomplished.

However, at least one observation was recorded for each animal during the first 4 hours after dosing. Pilot study results did not provide an adequate indication of the toxicity observed in the female animals. Consequently, an additional group was added to the female study so that a dose level with less than 50% mortality could be achieved. On analysis, the 683 mg/kg dosing solution was a 311-mg/kg solution. Since this was the low dose group and no mortalities had occurred, this group had no impact on the calculated MLD values. Steam outages occurred from 0700 to 1800 hours on 3 March 1984 and from 2200 hours on 22 March 1984 to 1400 hours on 23 March 1984. These days were selected by the building engineers for routine maintenance. During these outages, the relative humidity went up to 64% in room RS1418 and 74% in RS1408, and the temperature fell to 18.9°C in RS1409. These changes did not appear to have any effect on the outcome of the study.

Storage of Raw Data and Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Forty-six (74%) of the deaths occurred between 1 and 8 hours after dosing. An additional 13 (21%) deaths occurred between 8 and 24 hours after dosing. The remaining three animals were found dead on the morning of the second day (between 36 and 47 hours after dosing). Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis, and the equation for the probit regression line was: $Y = -19.6 + 8.2 \log X$ for males and $Y = -32.5 + 13.1 \log X$ for females, where X is the dose and Y the corresponding probit value. Misdosed animals were excluded from statistical analysis and eliminated from the study. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

TABLE 2

Guanidine Nitrate-Related Deaths by Group

GROUP	Dose Level (mg/kg)	Deaths/ Number in Group	Percent Mortality
		MALES	
1	685*	0/10	0
2	826	3/10	30
3	1000	3/8>	38
4	1210	7/8>	88
5	1470	9/10	90
6	Vehicle	0/5	0
		FEMALES	
6A	610	1/7>	14
1A	718	5/9>	56
2A	847	6/9>	67
3A	1000	10/10	100
4A	1180	10/10	100
5A	1390	8/8>	100
7	Vehicle	0/5	0

* Due to an error in the preparation of this solution these animals actually received 311 mg/kg.

> Reduced numbers in groups were due to one or more misdosed animals which were excluded from statistical analysis and removed from the study.

Clinical Observations

Guanidine nitrate produced clinical signs at each dose level. The most frequently observed signs were behavioral (63 of 99 animals dosed with test compound), gastrointestinal (GI) tract symptoms (37 of 99), and respiratory (26 of 99) signs. Most clinical signs were recorded within the first 48 hours, although one behavioral sign, irritability, was observed in a few animals intermittently throughout the study. However, irritability tended to decrease as dose levels increased, and it was also present in 7 of the 10 vehicle control animals.

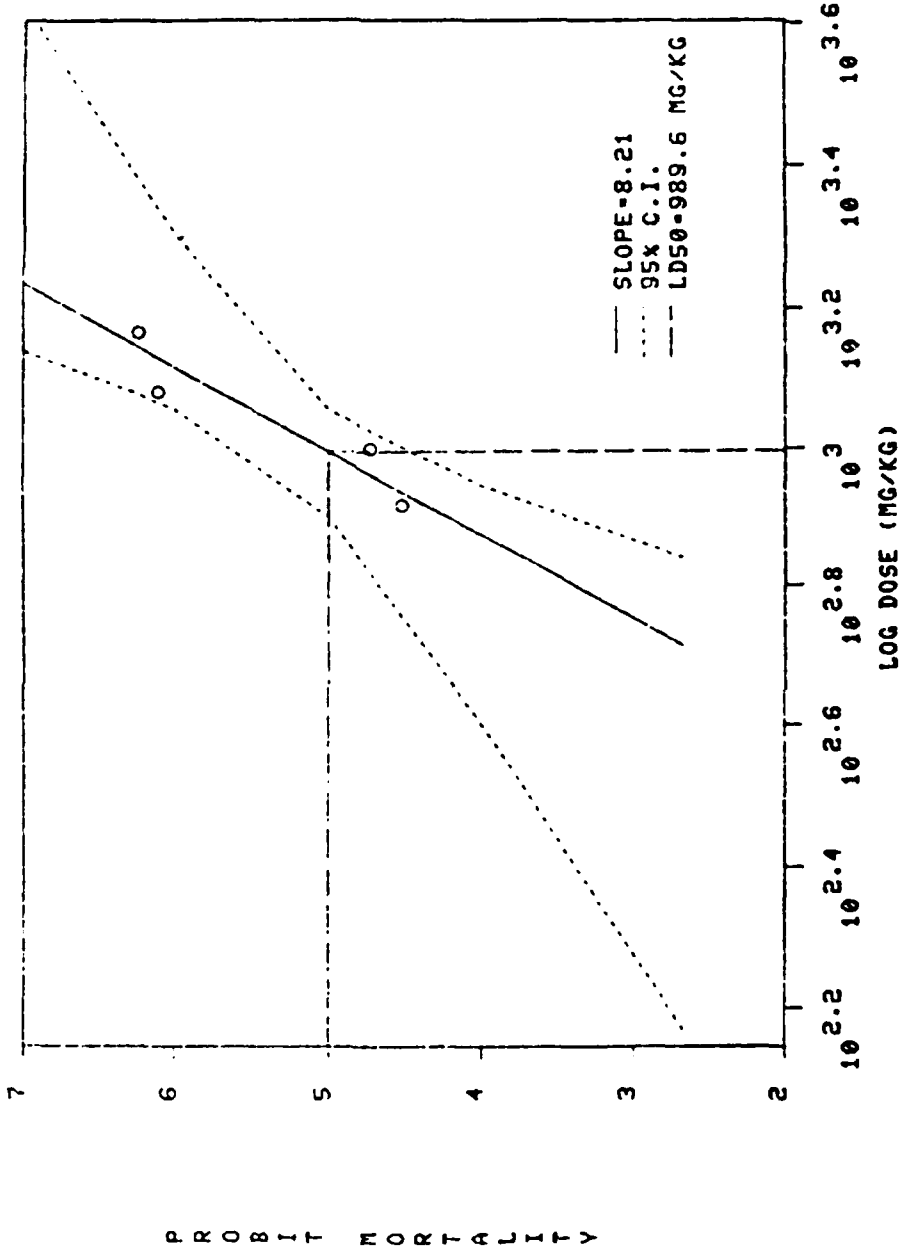


FIGURE 1: Dose Mortality Curve for Guanidine Nitrate in Male Rats

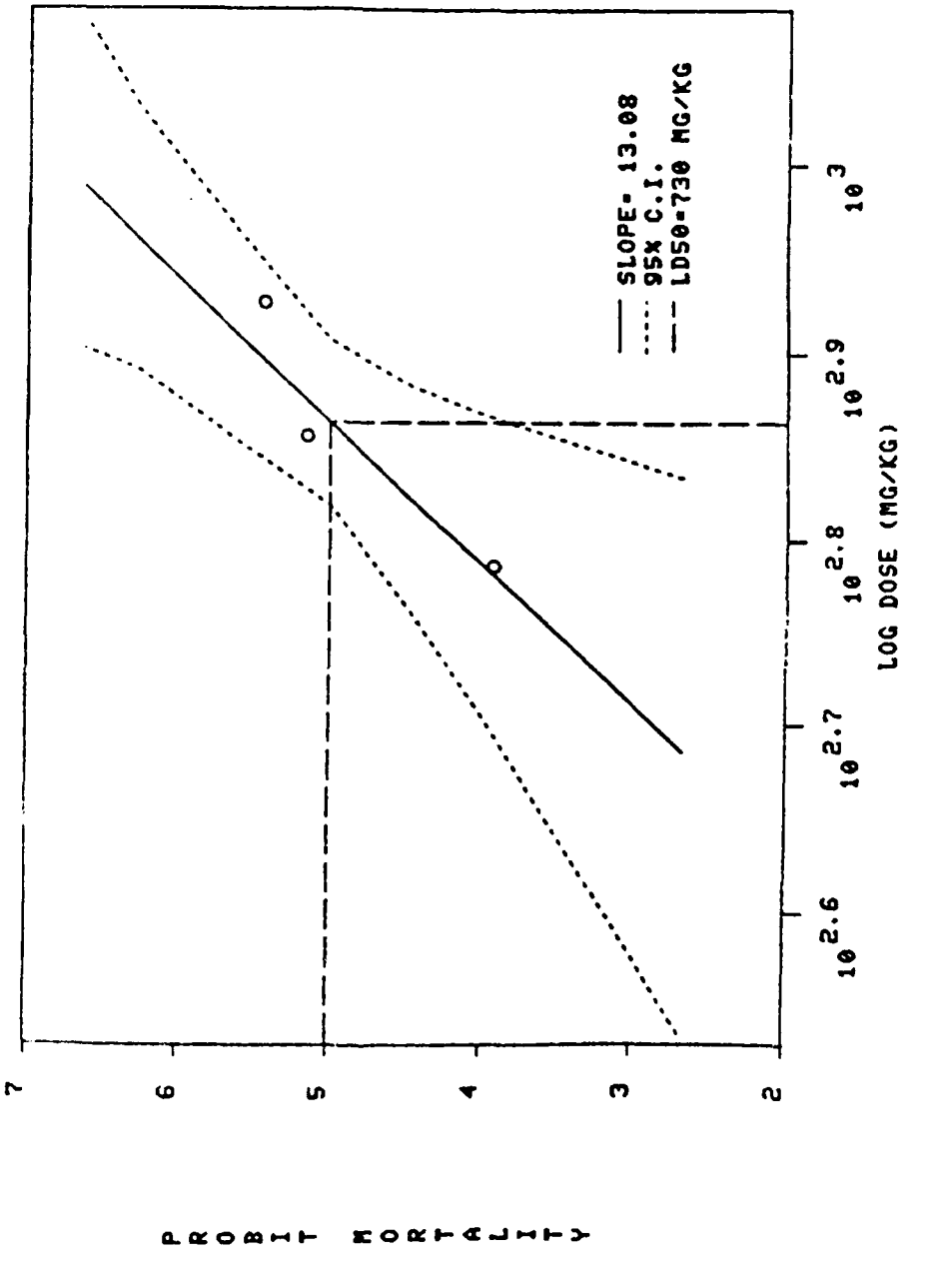


FIGURE 2: Dose Mortality Curve for Guanidine Nitrate in Female Rats

TABLE 3

Calculated Lethal Doses (LD) of Guanidine Nitrate

Effect Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
	MALES	
LD10	690.8 ± 104.5	(324.1, 838.7)
LD50	989.6 ± 68.7	(793.0, 1137.8)
LD90	1417.8 ± 166.0	(1210.8, 2473.6)
	FEMALES	
LD10	582.4 ± 50.9	(414.5, 657.1)
LD50	729.8 ± 34.3	(640.7, 799.4)
LD90	914.5 ± 60.3	(829.3,1161.4)

* Calculated dose ± standard error

Behavioral signs were present in all dose groups. A dose-response relationship was observed for this group of signs. The dose-response relationship is more apparent if one deletes those animals that died before clinical observation could be made. For the highest two female dose groups, all animals with recorded symptoms displayed one or more behavioral signs. Behavioral signs included ataxia, inactivity, changes in preening behavior, disorientation, irritableness, aggressiveness, hyperactivity, jumping, prostrate condition, tremors, and twitching.

GI symptoms included increased salivation, reddish material present in mouth, diarrhea, and perianal staining. These symptoms were more prevalent at the lower dose levels. The apparent lower frequencies of all these signs in the higher dose groups are attributed to the fact that these animals rapidly progressed to the more severe signs and/or died before any of the signs could be observed. Table 4 contains a summary of the clinical signs observed. Appendix E contains the individual animal histories. Weight gains of survivors were not significantly affected by dosing. Table 5 presents the mean body weights by groups. Appendix F contains weight tables for individual rats.

TABLE 4

Incidence of Clinical Signs in Rats Administered Guanidine Nitrate

MALES

Clinical Signs	Group (Dose) (n=)	1 Control 5	2 683 10	3 826 10	4 1000 8	5 1210 8	6 1470 10
Behavioral*		3	8	10	4	5	6
Gastrointestinal>		0	2	5	2	3	5
Respiratory†		2	1	4	0	1	4
Ocular<		0	0	2	0	0	2
Rough coat		0	0	1	0	0	0
Hunched posture		0	0	0	0	2	1
Normal throughout		1	2	0	0	0	0

FEMALES

Clinical Signs	Group (Dose) (n=)	1 Control 5	6A 610 7	1A 718 9	2A 847 9	3A 1000 10	4A 1180 10	5A 1390 8
Behavioral*		5	5	9	6	9	8	3
Gastrointestinal>		1	5	6	2	2	5	1
Respiratory†		1	4	2	4	5	0	1
Ocular<			0	1	0	1	0	0
Rough coat		0	1	5	3	1	2	1
Normal throughout		0	1	0	0	0	0	0

* Includes ataxia, disorientation, hyperactivity, jumping, irritableness, inactivity, changes in preening, prostrate condition, aggressiveness, somnolence, tremors, and twitching.

> Includes increased salivation, material in mouth, diarrhea, and perianal staining.

† Includes reddish nasal discharge and stains on head, increase in respiratory rate, and decrease in respiratory depth.

< Includes reddish material around eyes (chromodacryorrhea) and conjunctivitis.

Table 5

Mean Body Weights in Grams ± S.E.*

Dose	Receipt	Day 0	Day 6†	Day 14
		MALES		
683 mg/kg	152.4 ±3.7(10)	223.9 ±5.4(10)	287.0 ±6.8(10)	290.2 ±8.0(10)
826 mg/kg	159.5 ±3.6(10)	222.6 ±5.4(10)	253.7 ±8.1(7)	274.3 ±7.1(7)
1000 mg/kg	158.5 ±4.5(10)	224.9 ±5.6(10)	283.2 ±7.6(5)	289.8 ±6.1(5)
1210 mg/kg	149.3 ±4.2(10)	224.0 ±5.6(10)	251.0(1)	269.0(1)
1470 mg/kg	155.0 ±4.1(10)	226.1 ±4.8(10)	217.0(1)	222.0(1)
Vehicle	156.2 ±3.8(5)	235.8 ±4.6(5)	306.8 ±5.2(5)	314.4 ±8.4(5)
		FEMALES		
610 mg/kg	144.1 ±5.3(8)	146.5 ±4.3(8)	176.5 ±5.8(6)	191.8 ±4.8(6)
718 mg/kg	136.6 ±1.4(10)	154.8 ±3.1(10)	217.3 ±10.3(4)	209.0 ±8.9(4)
847 mg/kg	136.7 ±2.9(10)	152.9 ±3.0(10)	192.7 ±7.3(3)	182.7 ±15.3(3)
1000 mg/kg	141.1 ±3.4(10)	156.3 ±2.9(10)		
1180 mg/kg	138.9 ±2.9(10)	154.7 ±3.5(10)		
1390 mg/kg	140.6 ±2.6(10)	155.6 ±2.8(10)		
Vehicle	151.6 ±2.4(5)	178.8 ±4.1(5)	215.4 ±4.9(5)	207.4 ±4.1(5)

* Number in parenthesis = number of animals.

† For the females (except controls) this is Day 7.

Pathology Report

The presence of multiple red foci in the thymuses of the females was the only gross lesion attributable to the test compound. The presence of foci exhibited a dose-response relationship. The pathologist's report is presented in Appendix G.

DISCUSSION

The calculated MLD for guanidine nitrate in Sprague-Dawley rats was 989.6 ± 68.7 mg/kg for males and 729.8 ± 34.3 mg/kg for females. These MLD values are within the slightly toxic range (5). The major category of clinical signs observed was behavioral, which included inactive, irritable, disoriented, and hyperactive. These findings are consistent with an effect on the central nervous/neuromuscular system and the reported action of guanidine as a striated muscle stimulant (6).

Similar results were also reported for guanidine hydrochloride (7). However, guanidine hydrochloride produced a considerably more profound effect on the CNS-NM system (80 of 86) and GI tract (53 of 86) than did guanidine nitrate. The clinical signs produced by guanidine hydrochloride were not only observed more frequently but were also more severe than those produced by guanidine nitrate. The oral MLDs in male and female rats for the hydrochloride salt were 556.5 mg(base)/kg and 474.6 mg(base)/kg, respectively. For comparison, the oral MLDs in male and female rats for guanidine nitrate would be 478.6 mg (base)/kg and 352.9 mg(base)/kg, respectively. These values are relatively close to those obtained for the hydrochloride salt. Therefore, one can attribute the toxicity of these two salts to the guanidine base.

CONCLUSIONS

Guanidine nitrate is a "slightly toxic" compound that produces behavioral, gastrointestinal, and respiratory signs. Calculated MLD values were 989.6 + 68.7 mg/kg in male and 729.8 ± 34.3 mg/kg in female Sprague-Dawley rats.

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Appendix A: CHEMICAL DATA

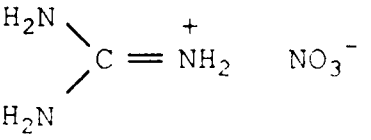
Chemical Name: Guanidine Nitrate

Lot Number: 123820

Chemical Abstracts Registry Number: 506-93-4

LAIR Code: TW030

Chemical Structure:



Molecular Formula: $CH_6N_3 \cdot NO_3$

Molecular Weight: 122.1

Physical State: White crystalline powder

Melting Point: 214°C[1]

Analytical Data:

Infrared spectrophotometry was performed and the spectrum obtained[2] was identical to the Sadtler spectrum[3] for Guanidine Nitrate. Major absorption peaks were observed at 3400 (broad), 3200, 1665, 1575, 1400, 1385, and 825 cm^{-1}. The grade of material obtained for this study is referred to as the Ultralog Grade by the manufacturer. The label on the bulk container states that the purity is at least 99.99%.

Source: Chemical Dynamics Corporation
Hadley Road, PO Box 395
South Plainfield, NJ

---

[1]Windholz M, ed., The Merck Index. 9th ed., Rahway, NJ: Merck and Co., Inc., 1976: Monograph Number 4414.

[2]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 62. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #14498.

Appendix A (cont.): CHEMICAL DATA

Analysis of Dosing Solutions/Suspensions and Determination of Stability

Dosing solutions/suspensions of guanidine nitrate were vortexed to ensure suspension of particulate material and 1 ml samples were removed from the top, middle, and bottom. Samples were transferred to screwcapped tubes and stored below 0°C prior to analysis

For analysis, dilution of the sample was necessary. The first dilution was accomplished by heating the 1 ml sample to 50°C in a water bath to dissolve suspended material, and then quickly cooling to room temperature. Before the guanidine nitrate could crystallize out of solution, 0.5 ml was transferred to a 10 ml volumetric flask and diluted to volume with water.

A second dilution of 1:100 was performed for a total dilution of 1:10,000. Aliquots (2 ml) of the final dilution were assayed using a modification of the Voges-Proskauer assay for guanidine.[1] Quantitation was accomplished by measuring the absorbance of a colored guanidine derivative at a wavelength of 535 nm.

For the first analysis, seven samples were chosen that represented the entire range of concentrations used for dosing.[2] The results indicated that homogenous suspensions can be prepared up to 34 mg/ml (Table 1). As a result of this determination, all subsequent analyses were performed with pooled samples (i.e., the top, middle, and bottom samples obtained from dosing solutions/suspensions were heated to dissolve suspended material and pooled).[3] These results are presented in Table 2. Of the ten suspensions analyzed, nine were determined to be within 6% of the target. The concentration of the 68-mg/ml solution, however, showed a deviation of 54.3% below the target value. This reflects, in all probability, a dilution error in making the solution.

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[1]Micklus MJ, Stein JM. The colorimetric determination of mono- and disubstituted guanidines. Anal Biochem 1973; 53:545-553.

[2]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-05-010.2, p 49-51, 56. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Ibid. p 52, 55-56.

Appendix A (cont.): CHEMICAL DATA

Analytical Method: Stock Solutions and Calibration Plot

Stock Solution - 50 µg/ml in water:

The stock solution was prepared by weighing out 50.0 mg of guanidine nitrate and transferring this amount to a 1000-ml volumetric flask. The compound was diluted to volume with water and mixed well.

Standard Curve (calibration plot):

To generate the standard curve, two ml of guanidine nitrate solution were prepared at a variety of concentrations as follows:

Final Concentration	ml of stock added to	mls of water
2 µg/ml	0.08 ml stock	1.92 ml water
5	0.20	1.80
10	0.40	1.60
15	0.60	1.40
20	0.80	1.20
25	1.00	1.00
30	1.20	0.80

Calculations:

Linear regression was used to calculate the standard curve. In all cases a correlation coefficient (r) of greater than 0.999 was obtained.

$$\text{assay value (mg/ml)} = \frac{\text{µg/ml found} \times \text{dilution factor}}{1000}$$

where µg/ml found is determined by linear regression

dilution factor = 10,000

Appendix A (cont.): CHEMICAL DATA

TABLE 1: Analysis of Dosing Suspensions for Homogeneity

Date Mixed	Date Analyzed	Source of Sample*	Target Conc. mg/ml		Actual Conc. mg/ml	% Target
6 Mar	18 May	T	66 >		67.4	102.1
		M			65.5	99.2
		B			65.7	99.5
				Mean	66.2	100.3
14 Mar	18 May	T	83 >		80.5	97.0
		M			75.7 <	91.2
		B			77.2	93.0
				Mean	77.8	93.7
8 Mar	18 May	T	100		96.0	96.0
		M			99.8	99.8
		B			97.6	97.6
				Mean	97.8	97.8
8 Mar	18 May	T	133		122.2	91.9
		M			131.2	98.6
		B			122.8	92.3
				Mean	125.4	94.3
8 Mar	18 May	T	166		162.5	97.9
		M			165.3	99.6
		B			158.7	95.6
				Mean	162.2	97.7
6 Mar	18 May	T	200		184.5	92.3
		M			188.0	94.0
		B			197.0	98.5
				Mean	189.8	94.9

*The letters T, M, and B refer to the top, middle, and bottom of the dosing solution/suspension.

>These samples were solutions.

<This sample was originally assayed on 18 May and a low value of 67.9 mg/ml was determined. Reanalysis on 29 May 84 gave a value of 75.7 mg/ml. As a check for consistency, the samples prepared on 6 Mar and 27 Mar were also reanalyzed on 29 May. The values obtained for these samples were within 1% of the respective values obtained on 18 May.

Appendix A (cont.): CHEMICAL DATA

TABLE 2: Verification of Guanidine Nitrate Concentrations*

Date Mixed	Date Analyzed	Target Conc. mg/ml	Actual Conc† mg/ml	% Target
14 Mar	6 Jun	68.0	31.0 ± 0.5	45.6
		83.0	79.7 ± 1.1	96.1
		100.0	93.8 ± 1.1	93.8
		121.0	113.3 ± 1.3	93.6
		147.0	137.9 ± 0.8	93.8
21 Mar	30 May	71.8	67.2 ± 1.3	93.6
		84.7	78.6 ± 0.2	92.8
		100.0	92.3 ± 0.2	92.3
		118.0	110.3 ± 0.6	93.5
		139.0	129.8 ± 0.2	93.4
27 Mar		61.0	not analyzed	

*Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 57-59. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†Mean and standard deviation of three analyses.

Appendix A (cont.): CHEMICAL DATA

Stability:

The stability of guanidine nitrate in aqueous solution is demonstrated by the absorbance values obtained for a standard solution containing 20 µg/ml of guanidine nitrate. This solution was prepared on 25 May and kept at room temperature over the period of analysis. From 25 May to 6 June, four assays of this solution were performed yielding statistically identical absorbance values.[1] Since the Voges-Proskauer assay is specific for unsubstituted and mono-substituted guanidines, the data demonstrate that aqueous solutions of guanidine nitrate are stable for a period of at least 12 days (Table 3).

TABLE 3: Stability Assay of a 20 µg/ml Standard Solution of Guanidine Nitrate

Date of Analysis	Absorbance Values*
25 May 84	1.74 ± 0.02
29 May 84	1.76 ± 0.05
30 May 84	1.76 ± 0.02
6 Jun 84	1.76 ± 0.02

* Values are mean ± S.D. for three replicates.

[1] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 55-57,59. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Rattus norvegicus*

Strain: Sprague-Dawley

Source: Bantin-Kingman
Fremont, CA

Sex: Male and Female

Date of Birth: Males: 23 January 1984
Females: 31 January 1984
8 February 1984 (for animals numbered 84D00-863, -865, -867, -868, -871, -886, -896, -900)

Method of Randomization: Weight bias, stratified animal allocation

Condition of animals at start of study: Normal

Body weight range at dosing: Males: 190 to 255 g
Females: 140 to 174 g

Identification Procedures: Ear tag. Tag numbers--Males: 84D00596-84D00667, Females: 84D00693-84D00794 and 84D00863-84D00900 with exclusions.

Pretest conditioning: Quarantine/acclimation. Males 29 Feb-7 Mar 84. Females 14-21 Mar 84, and 21-28 Mar 84 for the additional group.

Justification: The laboratory rat has been proven to be a sensitive and reliable system for determinations of lethal dose.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

FEMALES

Date	Event
29 Feb 84	Received initial shipment of animals. Five females were used for vehicle controls; their dates are the same as those of the events for males (next page).
20 Mar 84	Fifty-two female animals were received from GLP Study 84016. Animals were randomized and allocated to groups 1A-2A. Food was removed by 2200 hours.
21 Mar 84	Animals in Groups 1A-5A were weighed, dosed, and observed. All animals that died were submitted for necropsy.
22 Mar-10 Apr 84	All animals were observed daily for mortality and clinical signs.
23 Mar 84	Eight female animals, Group 6A, received from GLP Study 84015, constituted an additional dose group.
27 Mar 84	Food was removed from Group 6A by 2200 hours.
28 Mar 84	Animals in Groups 1A-5A were weighed. Group 6A animals were weighed, dosed, and observed. All animals that died were necropsied.
4 Apr 84	All surviving animals in groups 1A-5A were observed, weighed, sacrificed, and necropsies performed. Group 6A animals were weighed.
11 Apr 84	All surviving animals in Group 6A were observed, weighed, sacrificed, and necropsies performed.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

MALES

Date	Event
29 Feb 84	Seventy-two male Sprague-Dawley rats were received at LAIR. Rats were housed individually and their left ear was tagged. Animals were weighed and two animals were submitted for quality control necropsy.
5 Mar 84	Animals were randomized, divided into dose groups, and weighed.
14 Mar 84	Animals were weighed, dosed, and observed. All animals that died were submitted for necropsy.
14-28 Mar 84	All animals were observed daily for mortality and clinical signs.
20 Mar 84	All animals were weighed.
28 Mar 84	All surviving animals were observed, weighed, sacrificed, and necropsies performed.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing: Hours 1	2	4	6	8	Days 1	2	3	4	5	6	7-14
FEMALES													
610	7	0	1	1	1	1	1	1	1	1	1	1	1
718	9	0	0	1	1	1	3	5	5	5	5	5	5
847	9	1	1	4	4	4	6	6	6	6	6	6	6
1000	10	0	0	7	7	7	10	10	10	10	10	10	10
1180	10	0	2	9	10	10	10	10	10	10	10	10	10
1390	8	0	4	5	8	8	8	8	8	8	8	8	8
Vehicle	5	0	0	0	0	0	0	0	0	0	0	0	0
MALES													
683	10	0	0	0	0	0	0	0	0	0	0	0	0
826	10	0	0	1	2	2	2	3	3	3	3	3	3
1000	8	0	0	1	2	2	3	3	3	3	3	3	3
1210	8	0	0	2	2	3	7	7	7	7	7	7	7
1470	10	0	1	6	7	8	9	9	9	9	9	9	9
Vehicle	5	0	0	0	0	0	0	0	0	0	0	0	0

Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALES: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00605	Irritable	March 15	Slight
84D00606	None Observed	N/A	N/A
84D00622	Nasal Discharge, Red	March 22	Slight
84D00655	Irritable	March 15	Moderate
		March 16-28	Slight
	Nasal Discharge, Red	March 22	Slight
84D00665	Inactive	March 15	Moderate
	Irritable	March 15	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 683 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00597	Material Perianal, Brown	March 14	Slight
	Irritable	March 15,17, 22,23	Slight
84D00600	Material Mouth, Clear	March 14	Slight
	Inactive	March 15	Slight
84D00602	None Observed	N/A	N/A
84D00617	Irritable	March 15,16	Slight
84D00635	Irritable	March 15-19	Slight
		March 20,21	Moderate
		March 22-25	Slight
84D00636	Hyperactive	March 15	Slight
	Irritable	March 19,20	Slight
84D00642	Hyperactive	March 15	Slight
	Irritable	March 15	Slight
		March 16	Moderate
84D00645	Irritable	March 16,17	Slight
	Inactive	March 16	Slight
	Nasal Discharge, Red	March 22	Slight
84D00651	None Observed	N/A	N/A
84D00662	Inactive	March 15	Marked
		March 16,17	Slight
		March 20,21	Slight
	Irritable	March 15	Slight
	Increased Temperature	March 15	Marked

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 826 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00596	Material Mouth, Red	March 14	Slight
	Material Perianal, Yellow	March 14	Moderate
	Ataxia	March 14	Slight
	Somnolence	March 14	Slight
	Death	March 14	6 hrs
84D00611	Nasal Discharge, Red	March 15	Slight
	Decreased Preening	March 15	Moderate
	Stain Perianal, Yellow	March 15	Marked
	Irritable	March 15,16	Moderate
		March 19	Slight
84D00615	Inactive	March 16	Moderate
84D00618	Tremors	March 14	Slight
		March 15	Marked
	Ataxia	March 14	Slight
	Decreased Preening	March 15	Marked
	Chromodacryorrhea	March 15	Marked
	Conjunctivitis	March 15	Marked
	Increased Salivation	March 15	Marked
	Stain Perianal, Yellow	March 15	Marked
	Moribund	March 15	N/A
	Death	March 16	48 hrs
84D00632	Hyperactive	March 15	Marked
	Irritable	March 20,21	Slight
84D00633	Rough Coat	March 14	Slight
	Irritable	March 15	Moderate
	Inactive	March 16	Moderate
84D00634	Prostrate	March 14	N/A
	Decreased Resp. Rate	March 14	Slight
	Increased Resp. Depth	March 14	Slight
	Death	March 14	5 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 826 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00639	Material Perianal, Yellow	March 14	Slight
	Twitching	March 15	Marked
	Tremors	March 15	Marked
	Hyperactive	March 15	Moderate
	Material Head, Red	March 15	Marked
	Stain Perianal, Yellow	March 15	Marked
	Decreased Preening	March 15	Marked
		March 16	Moderate
84D00640	Irritable	March 15	Moderate
	Hyperactive	March 15	Slight
84D00643	Inactive	March 14	Slight
	Decreased Preening	March 15	Marked
	Tremors	March 15	Marked
	Chromodacryorrhea	March 15	Marked
	Material Head, Red	March 15	Marked
	Stain Perianal, Yellow	March 15	Moderate
	Irritable	March 16	Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 1000mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00598	Material Mouth, Red	March 14	Slight
84D00604	Material Mouth, Red	March 14	Slight
	Ataxia	March 14	Slight
	Inactive	March 16	Slight
84D00624	Irritable	March 15,16	Moderate
84D00625	Hyperactive	March 15	Moderate
	Irritable	March 16,17, 18,21	Slight
	Inactive	March 20	Slight
84D00627	Ataxia	March 14	Moderate
	Jumping	March 14	Marked
	Death	March 14	5.5 hrs
84D00631	None Observed	N/A	N/A
84D00637	Misdose		
84D00638	Ataxia	March 14	Slight
	Death	March 15	24 hrs
84D00658	Death	March 14	5 hrs
84D00659	Misdose		

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1210 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00607	Hunched Posture	March 15	Moderate
	Decreased Preening	March 15	Moderate
	Material Mouth, Red	March 15	Marked
	Material Perianal, Yellow	March 15	Slight
	Hyperactive	March 15	Moderate
84D00612	Hunched Posture	March 14	Slight
	Ataxia	March 14	Slight
	Material Mouth, Clear	March 14	Slight
	Death	March 15	24 hrs
84D00614	Prostrate	March 14	N/A
	Increased Resp. Rate	March 14	Slight
	Decreased Resp. Depth	March 14	Slight
	Death	March 14	7 hrs
84D00619	Death	March 15	24 hrs
84D00620	Death	March 14	5 hrs
84D00644	Death	March 14	3 hrs
84D00650	Misdose		
84D00654	Ataxia	March 14	Slight
	Inactive	March 14	Slight
	Death	March 15	24 hrs
84D00661	Material Perianal, Brown	March 14	Slight
	Material Right Hind Leg	March 14	Slight
	Ataxia	March 14	Slight
	Death	March 15	24 hrs
84D00664	Misdose		

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1470 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00601	Inactive	March 14	Moderate
	Material Mouth, Clear	March 14	Slight
	Increased Resp. Depth	March 14	Slight
	Decreased Resp. Rate	March 14	Slight
	Death	March 14	5.5 hrs
84D00609	Material Mouth, Clear	March 14	Moderate
	Inactive	March 14	Slight
	Death	March 14	3.5 hrs
84D00610	Material Mouth, Red	March 14	Slight
	Inactive	March 14	Slight
	Increased Resp. Depth	March 14	Slight
	Decreased Resp. Rate	March 14	Slight
	Death	March 14	3.5 hrs
84D00613	Material Mouth, Clear	March 14	Slight
	Inactive	March 14	Marked
	Material Eye, Red	March 14	Slight
	Twitching	March 14	Slight
	Ataxia	March 14	Moderate
	Death	March 14	7 hrs
84D00616	Material Mouth, Clear	March 14	Slight
	Inactive	March 14,20,21	Slight
	Hunched Posture	March 14	Marked
	Material Eye, Red	March 14	Slight
	Twitching	March 15	Marked
	Decreased Preening	March 15	Moderate
		March 16	Slight
	Nasal Discharge, Red	March 15	Moderate
	Stain Mouth, Red	March 15	Slight
	Feces Perianal	March 15	Moderate
	Irritable	March 16	Moderate
		March 17,19	Slight
	Material Perianal, Brown	March 16	Slight
84D00626	Increased Resp. Depth	March 14	Slight
	Death	March 14	2 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1470 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00630	Death	March 14	3.5 hrs
84D00649	Death	March 14	2 hrs
84D00656	Death	March 14	3.5 hrs
84D00663	Ataxia	March 14	Slight
	Death	March 15	24 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00693	Irritable	March 16	Slight
84D00695	Material Nose, Red	March 15	Marked
		March 16	Slight
	Increased Resp Rate	March 15	Marked
	Stain Perianal, Yellow	March 15	Marked
		March 16-18	Moderate
	Wheezing	March 15	Moderate
	Decreased Preening	March 16	Moderate
	Irritable	March 21	Slight
84D00706	Irritable	March 15	Marked
		March 17	Moderate
		March 18,19	Slight
84D00714	Hyperactive	March 15	Moderate
84D00737	Irritable	March 21	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 610 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00863	Inactive	March 28	Moderate
	Material Mouth, Clear	March 28	Moderate
	Disoriented	March 28	Slight
	Increased Resp. Depth	March 28	Moderate
	Decreased Resp. Rate	March 28	Moderate
	Twitching	March 28	Marked
	Moribund	March 28	N/A
	Death	March 28	2.5 hrs
84D00865	None Observed	N/A	N/A
84D00867	Material Mouth, Clear	March 28	Slight
84D00868	Inactive	March 28	Moderate
	Irritable	March 28	Moderate
	Decreased Resp. Rate	March 28	Slight
	Increased Resp. Depth	March 28	Slight
	Material Nose, Red	March 28	Slight
84D00871	Misdose		
84D00886	Inactive	March 28	Moderate
		April 1-3	Moderate
	Irritable	March 28,29	Moderate
		March 31,	Slight
		April 3	Slight
	Disoriented	March 28	Slight
		March 29	Marked
		March 30	Moderate
	Stain Mouth, Red	March 28	N/A
	Diarrhea	March 29	Marked
	Stain Perianal, Yellow	March 29,31	Marked
		March 30	Moderate
		April 5	Slight
	Stain Head, Red	March 29	Marked
		April 5	Slight
	Stain Head, Clear	March 30	Marked
	Rough Coat	March 30,31	Moderate
		April 1	Moderate
		April 2,3	Slight
	Increased Resp. Rate	April 1	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 610 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00896	Inactive	March 28	Moderate
	Irritable	March 28	Slight
	Stain Mouth, Red	March 28	N/A
	Excited	March 29	Moderate
84D00900	Disoriented	March 28	Slight
	Increased Resp. Rate	March 28	Slight
	Decreased Resp. Depth	March 28	Slight
	Stain Mouth, Red	March 28	Slight
	Aggressive	March 28	N/A
	Irritable	March 28,29	Slight
	Inactive	March 28	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 718 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00750	Aggressive	March 21	Slight
	Hyperactive	March 21	Slight
	Inactive	March 21	Marked
		March 22	Moderate
	Twitching	March 21	Slight
	Material Mouth, Red	March 22	Moderate
	Rough Coat	March 22	Moderate
	Increased Resp. Rate	March 22	Slight
	Decreased Resp. Rate	March 22	Slight
84D00751	Aggressive	March 21	Slight
	Irritable	March 21,22	Slight
	Disoriented	March 22	Slight
84D00753	Inactive	March 21	Slight
	Stain Mouth, Clear	March 21	Slight
	Death	March 21	4 hrs
84D00756	Disoriented	March 21	Slight
	Rough Coat	March 21	Slight
	Increased Salivation	March 21	Slight
	Twitching	March 21,22	Slight
	Stain Mouth, Clear	March 21,22	Marked
	Stain Head, Clear	March 21,22	Slight
	Material Perianal, Yellow	March 21,22	Moderate
	Material Abdomen, Yellow	March 21,22	Moderate
	Increased Resp. Rate	March 22	Slight
	Death	March 23	48 hrs
84D00760	Rough Coat	March 21,22	Moderate
	Stain Mouth, Red	March 21	Moderate
	Inactive	March 21	Moderate
		March 22	Marked
	Stain Mouth, Clear	March 22	Moderate
	Death	March 23	48 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 718 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00762	Hyperactive	March 21	Slight
	Irritable	March 21,22	Slight
	Inactive	March 22	Slight
84D00764	Hyperactive	March 21	Slight
	Twitching	March 21	Moderate
	Rough Coat	March 21	Moderate
	Material Mouth, Clear	March 21	Slight
	Death	March 22	24 hrs
84D00765	Hyperactive	March 21	Slight
	Inactive	March 21	Marked
	Twitching	March 21	Slight
	Death	March 22	24 hrs
84D00772	Misdose		
84D00793	Irritable	March 21	Moderate
		March 22	Slight
	Inactive	March 21	Marked
	Stain Mouth, Clear	March 21	Moderate
	Material Eye, Clear	March 21	Marked
	Rough Coat	March 22	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 847 mg/kg Guanidine Nitrate

Animal	Clinical Signs	Dates (1984)	Severity
84D00742	Death	March 21	4 hrs
84D00743	Death	March 21	4 hrs
84D00755	Irritable	March 21	Slight
	Death	March 21	4 hrs
84D00761	Rough Coat	March 21	Slight
	Increased Resp. Rate	March 21	Slight
	Hyperactive	March 21	Slight
	Inactive	March 21	Moderate
	Material Mouth, Red	March 21	Slight
	Death	March 22	24 hrs
84D00767	Misdose		
84D00770	Hyperactive	March 21	Slight
	Irritable	March 21,22	Slight
	Inactive	March 22	Slight
	Rough Coat	March 22	Slight
	Decreased Preening	March 22	Slight
	Increased Resp. Rate	March 22	Slight
84D00781	Disoriented	March 21	Slight
	Inactive	March 21	Slight
	Irritable	March 22	Slight
		April 1	Slight
	Stain Nose, Red	March 26	Slight
84D00782	Disoriented	March 21	Slight
	Inactive	March 21	Slight
	Material Mouth, Red	March 21	Slight
	Irritable	March 22	Slight
	Rough Coat	March 22	Slight
84D00788	Inactive	March 21	Slight
	Tremors	March 21	Slight
	Increased Resp. Rate	March 21	Slight
	Irritable	March 21	Moderate
	Death	March 22	24 hrs
84D00789	Death	March 21	1 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1000 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00747	Disoriented	March 21	Slight
	Hyperactive	March 21	Moderate
	Death	March 21	4.5 hrs
84D00749	Hyperactive	March 21	Slight
	Increased Resp. Depth	March 21	Moderate
	Death	March 21	4.5 hrs
84D00758	Moribund	March 21	N/A
	Death	March 21	4.5 hrs
84D00766	Increased Salivation	March 21	Marked
	Increased Resp. Rate	March 21	Slight
	Disoriented	March 21	Slight
	Death	March 21	4.5 hrs
84D00775	Inactive	March 21	Slight
	Disoriented	March 21	Moderate
	Increased Resp. Rate	March 21	Moderate
	Death	March 21	4.5 hrs
84D00777	Disoriented	March 21	Slight
	Hyperactive	March 21	Slight
	Irritable	March 21	Marked
	Inactive	March 21	Moderate
	Aggressive	March 21	Slight
	Death	March 22	24 hrs
84D00780	Irritable	March 21	Slight
	Inactive	March 21	Slight
	Increased Resp. Rate	March 21	Slight
	Death	March 21	4.5 hrs
84D00783	Inactive	March 21	Moderate
	Death	March 21	4.5 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1000 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00786	Disoriented	March 21	Slight
	Tremors	March 21	Slight
	Inactive	March 21	Slight
	Material Mouth, Clear	March 21	Slight
	Death	March 22	24 hrs
84D00787	Rough Coat	March 21	Slight
	Tremors	March 21	Slight
	Increased Resp. Rate	March 21	Slight
	Inactive	March 21	Slight
	Material Eye, Red	March 21	Slight
	Death	March 22	24 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1180 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00744	Death	March 21	2 hrs
84D00746	Irritable	March 21	Slight
	Disoriented	March 21	Slight
	Stain Mouth, Clear	March 21	Moderate
	Death	March 21	4.5 hrs
84D00748	Disoriented	March 21	Slight
	Hyperactive	March 21	Slight
	Death	March 21	4.5 hrs
84D00759	Rough Coat	March 21	Slight
	Increased Salivation	March 21	Moderate
	Disoriented	March 21	Slight
	Death	March 21	5 hrs
84D00768	Twitching	March 21	Slight
	Increased Salivation	March 21	Moderate
	Inactive	March 21	Moderate
	Death	March 21	4.5 hrs
84D00774	Disoriented	March 21	Slight
	Increased Preening	March 21	Slight
	Tremors	March 21	Moderate
	Inactive	March 21	Marked
	Material Mouth, Clear	March 21	Slight
	Death	March 21	6 hrs
84D00778	Hyperactive	March 21	Slight
	Rough Coat	March 21	Slight
	Death	March 21	5 hrs
84D00784	Disoriented	March 21	Slight
	Death	March 21	4.5 hrs
84D00785	Inactive	March 21	Slight
	Tremors	March 21	Moderate
	Increased Salivation	March 21	Moderate
	Death	March 21	4.5 hrs
84D00794	Death	March 21	2 hrs

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1390 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84D00745	Death	March 21	2.5 hrs
84D00754	Misdose		
84D00757	Death	March 21	1.5 hrs
84D00763	Rough Coat	March 21	Moderate
	Increased Salivation	March 21	Marked
	Disoriented	March 21	Moderate
	Death	March 21	5.5 hrs
84D00771	Death	March 21	1.5 hrs
84D00773	Death	March 21	2.5 hrs
84D00776	Hyperactive	March 21	Slight
	Increased Resp. Rate	March 21	Slight
	Death	March 21	4.5 hrs
84D00779	Misdose		
84D00790	Inactive	March 21	Marked
	Tremors	March 21	Marked
	Moribund	March 21	N/A
	Death	March 21	4.5 hrs
84D00791	Death	March 21	2 hrs

Appendix F: INDIVIDUAL BODY WEIGHTS

MALES: Vehicle Controls

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00605	158	233	304	311	78
606	152	229	293	294	65
622	146	230	307	309	79
655	156	233	305	313	80
665	169	254	325	345	91
Mean	156.2	235.8	306.8	314.4	78.6
Standard Deviation	8.5	10.3	11.5	18.7	9.2
Standard Error	3.8	4.6	5.2	8.4	4.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 683 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00597	151	210	260	270	60
600	175	255	321	323	68
602	168	242	304	311	69
617	144	206	264	257	51
635	144	218	294	293	75
636	160	217	272	265	48
642	137	205	261	262	57
645	147	219	287	289	70
651	152	242	305	316	74
662	146	225	302	316	91
Mean	152.4	223.9	287.0	290.2	66.3
Standard Deviation	11.8	17.0	21.6	25.4	12.8
Standard Error	3.7	5.4	6.8	8.0	4.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 826 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00596	167	253	Dead		
611	137	198	236	251	53
615	159	219	269	273	54
618	152	198	Dead		1
632	157	215	258	268	53
633	160	232	300	310	78
634	173	240	Dead		
639	151	228	256	282	54
640	175	227	280	275	48
643	164	216	247	261	45
Mean	159.5	222.6	263.7	274.3	55.0
Standard Deviation	11.3	17.1	21.4	18.7	10.7
Standard Error	3.6	5.4	8.1	7.1	4.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 1000 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00598	169	235	281	288	53
604	173	243	310	307	64
624	134	220	270	291	71
625	163	228	287	294	66
627	144	190	Dead		
631	164	225	268	269	44
637	158	217	Misdose		
638	159	222	Dead		
658	178	255	Dead		
659	143	214	Misdose		
Mean	158.5	224.9	283.2	289.8	59.6
Standard Deviation	11.2	17.6	16.9	13.7	10.9
Standard Error	4.5	5.6	7.6	6.1	4.9

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 1210 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00607	141	208	251	269	61
612	173	263	Dead		
614	138	215	Dead		
619	135	200	Dead		
620	162	224	Dead		
644	146	223	Dead		
650	145	237	Misdose		
654	149	234	Dead		
661	138	213	Dead		
664	166	223	Misdose		
Mean	149.3	224.0	251.0	269.0	61
Standard Deviation	13.2	17.7			
Standard Error	4.2	5.6			

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 1470 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00601	164	228	Dead		
609	167	242	Dead		
610	173	251	Dead		
613	171	239	Dead		
616	140	204	217	222	18
626	151	218	Dead		
630	150	229	Dead		
649	146	205	Dead		
656	137	222	Dead		
663	151	223	Dead		
Mean	155.0	226.1	217.0	222.0	18
Standard Deviation	12.9	15.2			
Standard Error	4.1	4.8			

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: Vehicle Controls

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00693	155	169	207	202	33
695	155	188	214	217	29
706	150	186	228	208	22
714	143	169	203	195	26
737	155	182	225	215	33
Mean	151.6	178.8	215.4	207.4	28.6
Standard Deviation	5.3	9.2	10.9	9.1	4.7
Standard Error	2.4	4.1	4.9	4.1	2.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 610 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00863	148	147	Dead		
865	137	140	169	185	45
867	147	150	195	204	54
868	116	124	156	173	49
871	150	148	Misdose		
886	165	167	170	196	29
896	156	152	183	190	38
900	134	144	186	203	69
Mean	144.7	146.5	176.5	191.8	45.7
Standard Deviation	15.0	12.1	14.1	11.8	10.9
Standard Error	5.3	4.3	5.8	4.8	4.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 718 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00750	138	144	189	184	40
751	145	171	232	219	48
753	138	154	Dead		
756	141	157	Dead		
760	134	141	Dead		
762	133	171	233	224	53
764	139	154	Dead		
765	135	153	Dead		
772	131	148	Misdose		
793	132	155	215	209	54
Mean	136.6	154.8	217.3	209.0	48.8
Standard Deviation	4.4	9.9	20.6	17.8	6.4
Standard Error	1.4	3.1	10.3	8.9	3.2

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 847 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00742	142	160	Dead		
743	131	163	Dead		
755	140	149	Dead		
761	125	144	Dead		
767	137	149	Misdose		
770	127	137	178	172	35
781	127	145	200	188	43
782	139	156	200	188	32
788	151	166	Dead		
789	148	160	Dead		
Mean	136.7	152.9	192.7	182.7	36.7
Standard Deviation	9.0	9.5	12.7	9.2	5.7
Standard Error	2.9	3.0	7.3	5.3	3.3

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1000 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00747	144	157	Dead		
749	138	160	Dead		
758	146	156	Dead		
766	143	158	Dead		
775	156	163	Dead		
777	123	145	Dead		
780	127	142	Dead		
783	156	174	Dead		
786	141	157	Dead		
787	137	151	Dead		
Mean	141.1	156.3			
Standard Deviation	10.7	9.0			
Standard Error	3.4	2.9			

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1180 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D0744	131	142	Dead		
746	141	159	Dead		
748	135	153	Dead		
759	133	148	Dead		
768	142	157	Dead		
774	123	139	Dead		
778	141	152	Dead		
784	151	175	Dead		
785	138	154	Dead		
794	154	168	Dead		
Mean	138.9	154.7			
Standard Deviation	9.2	10.9			
Standard Error	2.9	3.5			

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1390 mg/kg

Animal Number	At Receipt (g)	Dosing (g)	Day 6 (g)	Termination Day 14 (g)	Weight Change (g)
84D00745	144	164	Dead		
754	144	158	Misdose		
757	136	154	Dead		
763	146	161	Dead		
771	131	146	Dead		
773	158	171	Dead		
776	141	155	Dead		
779	140	159	Misdose		
790	132	146	Dead		
791	134	142	Dead		
Mean	140.6	155.6			
Standard Deviation	8.1	9.0			
Standard Error	2.6	2.8			

Appendix G: PATHOLOGY REPORT

Pathology Report

GLP Study 84-001

Oral Lethal Dose (LD_{50}) Test in Rats

of Guanidine Nitrate ($CH_5N_3.HNO_3$) ((AS No. 506-93-4)

History:

Fifty-three male and 59 female Sprague-Dawley rats, 6 weeks of age, were placed in the following test groups:

Group No.	Dose mg/kg	No. Rats	Sex
1	Vehicle Control	5	male
2	683	10	male
3	826	10	male
4	1000	10	male
5	1210	8	male
6	1470	10	male
1	Vehicle Control	5	female
1A	718	9	female
2A	847	9	female
3A	1000	10	female
4A	1180	10	female
5A	1390	9	female
6A	610	7	female

The test compound was dissolved in sterile water and dosed by oral gavage. Forty-nine rats died and were necropsied on the dosing day. An additional 14 rats died within 24 hours, and 2 died within 48 hours. The remaining rats survived until completion of the study and were killed by intraperitoneal injection of sodium pentobarbital.

Gross Necropsy Findings:

Individual animal data and group data are separated in Tables I and II. Iatrogenic deaths due to esophageal preforation or tracheal tears and deposition of the test substance in the thorax occurred in 2 males and 1 female. Thirteen females had multiple red foci in the thymus. None of the males had this lesion.

Summary:

Sixty-five rats (24 males, 41 females) died during testing. Three rats, 2 males and 1 female, were inappropriately dosed resulting in iatrogenic deaths. Therefore, 62 rats can be considered to have died as a result of the test compound. The only lesions attributable to the compound were multiple red foci in the thymus. This was confined to

Appendix G (cont.): PATHOLOGY REPORT

Pathology Report - GLP Study 84-001

the females and appeared to be dose dependent. The remaining lesions were considered incidental findings which were not compound related. No lesions were seen in the vehicle control rats.

LANCE O. LOLLINI, DVM
Diplomate, ACVP
LTV, VC
Chief, Pathology Services Group

Appendix G (cont.): PATHOLOGY REPORT

Table I

Oral LD_{50} in Rats (CAS No. 506-93-4)

GLP Study No. 84001

Females

ID #8480-XXX	Group 1	Group 1A	Group 2A	Group 3A
	6 6 7 7 7	7 7 7 7 7 7 7 7 7 7	7 7 7 7 7 7 7 7 7 7	7 7 7 7 7 7 7 7 7 7
	0 0 0 1 1	5 6 6 5 6 5 5 6 9	4 4 5 8 6 8 7 8 8	4 4 5 6 7 7 8 8 6 8
	3 5 6 7 7	1 4 5 6 0 0 1 2 3	2 3 5 9 1 8 0 1 2	7 9 8 6 5 7 0 3 6 7
Survival to Completion	+ + + + +	0 0 0 0 0 + + + +	0 0 0 0 0 + + + +	0 0 0 0 0 0 0 0 0 0
Thymus Multiple red foci	0 0 0 0 0	+ 0 0 0 0 0 0 0 0	0 0 0 + 0 0 0 0 0	0 0 0 0 0 0 + 0 0 0
Lung Red focus	0 0 0 0 0	0 + 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Small Intestine Luminal Contents Dark red	0 0 0 0 0	0 0 0 + + 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Blood Brown	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 + 0 0 0 0 0 0
Stomach - red foci multiple	0 0 0 0 0	0 0 0 + 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Luminal contents - dark red	0 0 0 0 0	0 0 0 + 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Esophagus Perforation	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Thorax Clear fluid present	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Bladder wall thickened	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Calculi	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Kidney Bilateral dilated pelvis	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Ureter - bilateral dilated	0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0

Appendix G (cont.): PATHOLOGY REPORT

Table IA

Oral LD_{50} in Rats (CAS No. 506-93-4)

GLP Study No. 84001

Females

ID #84D00XXX	Group 4A	Group 5A	Group 6A
	7 7 7 7 7 7 7 7 7 7 4 4 4 5 6 7 7 8 8 9 4 6 8 9 8 4 8 4 5 4	7 7 7 7 7 7 7 7 7 7 4 5 6 7 7 7 7 9 9 9 5 7 3 1 3 6 9 0 1	8 8 8 8 8 8 9 6 6 6 6 8 9 0 3 5 7 8 6 6 0
Survival to Completion	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 + + + + + +
Thymus Multiple red foci	0 0 0 + 0 + + 0 0 +	0 + 0 + + 0 + 0 +	0 0 0 0 0 0 0
Lung Red focus	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0
Small Intestine Luminal Contents Dark red	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0
Blood Brown	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0
Stomach - red foci multiple	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0
luminal contents - dark red	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0
Esophagus Perforation	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 + 0 0	0 0 0 0 0 0 0
Thorax Clear fluid present	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 + 0 0	0 0 0 0 0 0 0
Bladder wall thickened	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 + 0
Calculi	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 + 0
Kidney Bilateral dilated pelvis	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 + 0
Ureter Bilateral dilated	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 + 0

Appendix G (cont.): PATHOLOGY REPORT

Table IB

Oral LD_{50} in Rats (CAS No. 506-93-4)

GLP Study No. 84001

Males

ID 84-D00XXX	Group 1	Group 2	Group 3
	6 6 6 6 6	5 6 6 6 6 6 6 6 6 6	5 6 6 6 6 6 6 6 6 6
	0 0 2 6 5	9 0 0 1 3 3 4 4 5 6	9 3 1 1 1 3 3 3 4 4
	5 6 2 5 5	7 0 2 7 5 6 2 5 1 2	6 4 8 1 5 2 3 9 0 3
Survival to Completion	+ + + + +	+ + + + + + + + + +	0 0 0 + + + + + + +
Blood - brown	0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	+ 0 0 0 0 0 0 0 0 0
Esophageal Perforation	0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Tracheal tear	0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Thorax fluid	0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Renal pelvs dilated unilateral	0 0 0 0 0	0 + 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Cortical cysts	0 0 0 0 0	0 0 + 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Eye unilateral peripheral corneal opacity	0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0

Appendix G (cont.): PATHOLOGY REPORT

Table I-B (Cont)

Oral LD_{50} in Rats (CAS No. 506-93-4)

GLP Study No. 84001

Males

ID #84D00XXX	Group 4	Group 5	Group 6
	6 6 6 6 6 5 6 6 6 6 3 2 5 5 3 9 0 2 2 3 7 7 8 9 8 8 4 4 5 1	6 6 6 6 6 6 6 6 1 2 4 1 1 5 6 0 4 0 4 9 2 4 1 7	6 6 6 6 6 6 6 6 6 6 0 0 1 1 2 3 4 5 6 1 1 9 0 3 6 0 9 6 3 6
Survival to Completion	0 0 0 0 0 + + + + +	0 0 0 0 0 0 0 +	0 0 0 0 0 0 0 0 0 +
Blood - brown	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Esophageal Perforation	+ 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Tracheal tear	0 0 0 + 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Thorax fluid	+ 0 0 + 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Renal pelivs dilated unilateral	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Cortical cysts	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0
Eye unilateral peripheral corneal opacity	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 + 0	0 0 0 0 0 0 0 0 0 0

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Table II

Oral LD_{50} in Rats (CAS No. 506-93-4) - GLP Study No. 84001

Group Data - Males

Group	1	2	3	4	5	6
No. Animals/Group	5	10	10	10	8	10
Dose mg/kg	Vehicle Control	683	826	1000	1210	1470
Died	0	0	3	5	7	9
Lesions-thymus multiple red foci	0	0	0	0	0	0
Lung red focus	0	0	0	0	0	0
Small intestine red contents	0	0	0	0	0	0
Blood - brown	0	0	1	0	0	0
Stomach red foci	0	0	0	0	0	0
Red contents	0	0	0	0	0	0
Esophageal preforation	0	0	0	1	0	0
Tracheal tear	0	0	0	1	0	0
Thoracic fluid	0	0	0	2	0	0
Bladder wall thickened	0	0	0	0	0	0
Calculi	0	0	0	0	0	0
Kidney - dilated pelvis bilateral	0	0	0	0	0	0
Unilateral	0	1	0	0	0	0
Cysts	0	1	0	0	0	0
Ureter dilated bilateral	0	0	0	0	0	0
Eye corneal opacity	0	0	0	0	1	0

Appendix G (cont.): PATHOLOGY REPORT

Table II (Cont)

Oral LD_{50} in Rats (CAS No. 500-93-4) - GLP Study No. 84001

Group Data - Females

Group	1	1A	2A	3A	4A	5A	6A
No. Animals/Group	5	9	9	10	10	9	7
Dose mg/kg	Vehicle Control	718	847	1000	1180	1390	610
Died	0	5	6	10	10	9	1
Lesions-thymus multiple red foci	0	1	1	2	4	5	0
Lung red focus	0	1	0	0	0	0	1
Small intestine red contents	0	2	0	0	0	0	0
Blood - brown	0	0	0	1	0	0	0
Stomach red foci	0	1	0	0	0	0	0
Red contents	0	1	0	0	0	0	0
Esophageal preforation	0	0	0	0	0	1	0
Tracheal tear	0	0	0	0	0	0	0
Thoracic fluid	0	0	0	0	0	1	0
Bladder wall thickened	0	0	0	0	0	0	1
Calculi	0	0	0	0	0	0	1
Kidney - dilated pelvis bilateral	0	0	0	0	0	0	1
Unilateral	0	0	0	0	0	0	0
Cysts	0	0	0	0	0	0	0
Ureter dilated bilateral	0	0	0	0	0	0	1
Eye corneal opacity	0	0	0	0	0	0	0

Distribution List

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